

Table S1: antibodies used for flow cytometry analysis

marker	fluorochrome	clone	supplier
CD45	VioGreen	REA747	Miltenyi Biotec
CD3	AF700	OKT3	Biolegend
CD4	PE-Vio770	REA623	Miltenyi Biotec
CD8	BV570	RPA-T8	Biolegend
HLA-DR	BV605	L243	Miltenyi Biotec
CD19	PerCP-Vio700	REA675	Miltenyi Biotec
CD14	VioBlue	REA599	Miltenyi Biotec
CD16	BV650	3G8	Biolegend
CD123	APC-Vio770	REA918	Miltenyi Biotec
CD56	PE	REA614	Miltenyi Biotec
CD25	VioBright515	REA945	Miltenyi Biotec
CD127	APC	REA614	Miltenyi Biotec
CD19	PerCP-Vio700	REA675	Miltenyi Biotec
CD27	VioBright515	REA499	Miltenyi Biotec
CD38	PE	REA671	Miltenyi Biotec
CD24	VioBlue	REA832	Miltenyi Biotec
CD5	APC-Vio770	REA782	Miltenyi Biotec
CD1d	APC	51,1	Miltenyi Biotec

BV = Brilliant Violet, AF = Alexa Fluor, PE = phycoerythrin, APC = allophycocyanin.

Table S2: circulating immune cells, absolute values, measured by Sysmex

Cell type	Baseline (mean \pm SD)	27h (mean \pm SD)	4d (mean \pm SD)	9d (mean \pm SD)
Leukocytes (*10 ⁹ /L)				
Young placebo	5.624 \pm 0.958	5.487 \pm 0.828	4.837 \pm 0.919	4.849 \pm 0.982
Old placebo	5.749 \pm 1.667	6.001 \pm 1.931	5.674 \pm 1.727	5.730 \pm 1.903
Young HCQ	5.218 \pm 1.330	5.150 \pm 1.118	4.847 \pm 1.099	4.731 \pm 0.991
Old HCQ	5.363 \pm 1.105	5.531 \pm 1.277	5.249 \pm 0.968	5.090 \pm 1.076
Lymphocytes (*10 ⁹ /L)				
Young placebo	1.940 \pm 0.312	2.101 \pm 0.284	1.833 \pm 0.306	1.618 \pm 0.317
Old placebo	1.647 \pm 0.596	1.952 \pm 0.843	1.663 \pm 0.668	1.689 \pm 0.621
Young HCQ	1.720 \pm 0.254	1.844 \pm 0.299	1.751 \pm 0.286	1.568 \pm 0.200
Old HCQ	1.474 \pm 0.236	1.578 \pm 0.323	1.568 \pm 0.221	1.403 \pm 0.275
Monocytes (*10 ⁹ /L)				
Young placebo	0.515 \pm 0.068	0.441 \pm 0.064	0.438 \pm 0.090	0.424 \pm 0.101
Old placebo	0.618 \pm 0.184	0.546 \pm 0.208	0.548 \pm 0.122	0.518 \pm 0.105
Young HCQ	0.481 \pm 0.147	0.412 \pm 0.124	0.439 \pm 0.120	0.416 \pm 0.097
Old HCQ	0.481 \pm 0.139	0.433 \pm 0.124	0.425 \pm 0.081	0.409 \pm 0.102
Neutrophils (*10 ⁹ /L)				
Young placebo	3.019 \pm 0.921	2.823 \pm 0.592	2.422 \pm 0.710	2.670 \pm 0.828
Old placebo	3.257 \pm 0.943	3.281 \pm 0.936	3.247 \pm 1.043	3.299 \pm 1.379
Young HCQ	2.794 \pm 1.035	2.696 \pm 0.811	2.434 \pm 0.874	2.504 \pm 0.821
Old HCQ	3.262 \pm 0.975	3.389 \pm 1.028	3.097 \pm 0.850	3.137 \pm 0.862

Table S3: circulating immune cells, absolute values, measured by flow cytometry

Cell type	Baseline (mean ± SD)	3h (mean ± SD)	27h (mean ± SD)	4d (mean ± SD)	9d (mean ± SD)
CD14 ⁺ monocytes (*10 ⁶ /L)					
Young placebo	324.9 ± 65.4	349.3 ± 86.8	341.4 ± 58.7	342.9 ± 61.1	375.0 ± 101.2
Old placebo	403.2 ± 138.0	470.7 ± 133.7	450.9 ± 209.8	479.5 ± 87.1	478.0 ± 119.5
Young HCQ	289.2 ± 108.7	328.7 ± 105.1	321.9 ± 120.6	335.8 ± 113.8	343.9 ± 84.9
Old HCQ	314.6 ± 117.7	371.8 ± 93.1	386.3 ± 111.0	363.4 ± 100.3	365.9 ± 129.0
CD19 ⁺ B cells (*10 ⁶ /L)					
Young placebo	220.4 ± 68.3	264.6 ± 105.5	294.9 ± 93.0	249.8 ± 91.4	217.5 ± 73.0
Old placebo	173.3 ± 102.5	193.8 ± 108.2	191.1 ± 87.6	207.0 ± 102.8	204.5 ± 91.2
Young HCQ	175.1 ± 103.4	211.2 ± 93.0	224.0 ± 106.5	219.1 ± 105.4	197.8 ± 90.2
Old HCQ	191.2 ± 66.6	192.8 ± 72.9	224.6 ± 112.4	205.6 ± 85.2	208.1 ± 86.3
CD3 ⁺ T cells (*10 ⁶ /L)					
Young placebo	1280 ± 261	1384 ± 377	1499 ± 261	1308 ± 212	1220 ± 246
Old placebo	1296 ± 565	1370 ± 597	1446 ± 726	1345 ± 633	1424 ± 666
Young HCQ	1186 ± 279	1204 ± 226	1373 ± 305	1267 ± 233	1215 ± 215
Old HCQ	1081 ± 241	1158 ± 228	1233 ± 319	1181 ± 233	1115 ± 194
CD4 ⁺ T cells (*10 ⁶ /L)					
Young placebo	711.1 ± 216.7	733.8 ± 230.7	815.1 ± 187.1	706.0 ± 146.5	672.7 ± 148.5
Old placebo	864.9 ± 367.3	886.2 ± 363.5	951.4 ± 413.4	883.1 ± 375.5	944.1 ± 457.8
Young HCQ	688.2 ± 215.3	674.5 ± 183.4	763.1 ± 202.0	701.2 ± 179.4	687.0 ± 169.2
Old HCQ	756.9 ± 195.9	788.5 ± 185.9	865.0 ± 295.2	806.8 ± 150.1	766.4 ± 160.8
CD8 ⁺ T cells (*10 ⁶ /L)					
Young placebo	487.2 ± 93.1	551.9 ± 162.8	577.7 ± 106.8	507.5 ± 98.7	463.1 ± 123.6
Old placebo	402.9 ± 271.9	447.0 ± 302.0	453.4 ± 367.4	433.5 ± 300.3	444.6 ± 301.1
Young HCQ	424.6 ± 93.3	454.3 ± 98.6	517.1 ± 151.4	486.2 ± 113.1	446.3 ± 78.7
Old HCQ	286.9 ± 106.4	331.5 ± 143.9	328.2 ± 107.5	335.5 ± 178.3	315.1 ± 153.8

Table S4: circulating immune cells, relative values, measured with flow cytometry

Cell type	Baseline (mean ± SD)	3h (mean ± SD)	27h (mean ± SD)	4d (mean ± SD)	9d (mean ± SD)
CD3 (% of CD45)					
Young placebo	42.77 ± 11.74	42.08 ± 10.28	45.33 ± 12.10	41.72 ± 14.22	45.04 ± 14.44
Old placebo	59.65 ± 7.50	55.73 ± 7.56	58.95 ± 7.35	59.07 ± 9.01	58.67 ± 10.21
Young HCQ	40.03 ± 9.64	41.67 ± 13.36	37.72 ± 12.77	38.63 ± 9.85	42.37 ± 8.51
Old HCQ	61.09 ± 8.33	59.68 ± 7.61	59.62 ± 7.31	62.27 ± 6.29	59.18 ± 7.66
CD4 (% of CD45)					
Young placebo	23.21 ± 8.34	22.20 ± 7.13	25.20 ± 8.08	22.52 ± 9.55	24.85 ± 8.94
Old placebo	40.35 ± 7.30	36.25 ± 6.29	39.39 ± 5.82	39.24 ± 7.97	39.02 ± 8.09
Young HCQ	22.85 ± 7.84	22.35 ± 8.38	21.31 ± 9.26	21.26 ± 7.74	23.84 ± 7.12
Old HCQ	42.71 ± 7.30	41.05 ± 6.27	41.14 ± 6.57	42.88 ± 6.05	40.82 ± 6.06
CD8 (% of CD45)					
Young placebo	13.42 ± 3.12	13.94 ± 2.32	13.56 ± 2.98	13.55 ± 1.33	14.64 ± 1.61
Old placebo	15.75 ± 7.37	15.87 ± 8.04	15.91 ± 7.64	15.84 ± 7.46	15.86 ± 8.62
Young HCQ	12.567 ± 2.743	13.61 ± 4.27	12.08 ± 3.65	12.50 ± 3.15	13.63 ± 2.27
Old HCQ	14.49 ± 7.12	14.69 ± 7.44	14.47 ± 7.04	16.16 ± 6.89	15.21 ± 7.23
Treg (% of CD4)					
Young placebo	6.046 ± 1.053	5.461 ± 2.124	5.888 ± 0.794	5.544 ± 2.096	5.734 ± 0.815
Old placebo	7.494 ± 3.206	7.725 ± 2.983	7.667 ± 2.468	7.530 ± 2.452	7.599 ± 2.684
Young HCQ	6.410 ± 1.476	6.611 ± 1.293	6.096 ± 1.052	5.958 ± 2.259	6.884 ± 0.852
Old HCQ	6.172 ± 1.561	6.160 ± 1.655	6.588 ± 1.842	6.340 ± 1.681	6.739 ± 1.870
NK (% of CD45)					
Young placebo	10.15 ± 6.05	10.91 ± 5.17	9.82 ± 4.69	9.51 ± 5.14	9.20 ± 4.51
Old placebo	13.78 ± 3.66	16.61 ± 3.34	15.00 ± 3.71	13.95 ± 3.00	14.44 ± 4.34
Young HCQ	8.15 ± 3.03	8.69 ± 3.56	7.48 ± 3.32	7.55 ± 3.10	8.25 ± 2.38
Old HCQ	13.02 ± 5.20	13.40 ± 5.79	13.26 ± 6.47	11.86 ± 5.70	11.93 ± 5.35
B cells (% of CD45)					
Young placebo	9.25 ± 2.90	9.82 ± 3.66	9.58 ± 3.46	9.03 ± 3.45	9.49 ± 2.89
Old placebo	9.13 ± 2.71	9.12 ± 2.64	10.26 ± 3.07	10.10 ± 3.07	9.96 ± 3.33
Young HCQ	7.36 ± 3.43	8.33 ± 3.48	8.27 ± 3.31	7.67 ± 3.49	8.29 ± 3.55
Old HCQ	11.45 ± 3.19	10.84 ± 3.82	11.21 ± 2.87	10.71 ± 2.70	11.26 ± 3.54
Breg (CD5 ⁺ CD1d ^{hi})					
Young placebo	0.599 ± 0.236	0.759 ± 0.490	0.599 ± 0.219	0.625 ± 0.184	0.646 ± 0.160
Old placebo	0.720 ± 0.238	0.519 ± 0.189	0.973 ± 0.538	0.783 ± 0.194	0.972 ± 0.955
Young HCQ	0.748 ± 0.339	1.229 ± 1.230	1.065 ± 0.624	0.755 ± 0.245	0.934 ± 0.514
Old HCQ	0.565 ± 0.197	0.548 ± 0.225	0.654 ± 0.236	0.855 ± 0.429	0.879 ± 0.698
Transitional B cells					
Young placebo	4.044 ± 2.204	4.295 ± 2.368	5.113 ± 2.223	4.736 ± 2.041	4.810 ± 2.216
Old placebo	4.014 ± 1.502	4.025 ± 1.520	4.223 ± 1.542	4.152 ± 1.331	3.849 ± 1.270
Young HCQ	3.900 ± 2.184	4.114 ± 2.234	4.848 ± 2.486	4.571 ± 1.819	4.576 ± 1.993
Old HCQ	4.131 ± 1.534	4.120 ± 1.606	4.375 ± 1.598	4.276 ± 1.577	4.341 ± 1.669
ASC					
Young placebo	0.955 ± 0.659	0.789 ± 0.446	0.660 ± 0.713	0.751 ± 0.502	1.135 ± 0.684
Old placebo	2.155 ± 3.520	1.359 ± 1.451	2.785 ± 4.223	1.205 ± 0.731	1.472 ± 1.006
Young HCQ	1.161 ± 0.780	1.197 ± 0.908	1.276 ± 1.078	1.304 ± 0.757	1.431 ± 1.021
Old HCQ	0.701 ± 0.439	0.618 ± 0.514	1.376 ± 0.607	0.971 ± 0.255	1.036 ± 0.818
Classical monocyte (% of CD45)					
Young placebo	2.033 ± 1.778	2.063 ± 1.222	2.205 ± 1.604	1.885 ± 1.481	2.276 ± 1.790
Old placebo	1.690 ± 0.926	2.095 ± 0.952	1.963 ± 0.969	2.219 ± 1.852	2.476 ± 1.276
Young HCQ	1.439 ± 1.308	1.699 ± 1.002	1.209 ± 1.181	1.579 ± 1.057	1.765 ± 1.276
Old HCQ	1.022 ± 0.588	1.131 ± 0.349	1.256 ± 0.613	0.920 ± 1.530	1.511 ± 0.693

Intermediate monocyte (% of CD45)					
Young placebo	0.377 ± 0.294	0.523 ± 0.484	0.503 ± 0.325	0.267 ± 0.131	0.546 ± 0.471
Old placebo	1.023 ± 0.527	0.983 ± 0.507	1.054 ± 0.488	0.825 ± 0.438	0.714 ± 0.370
Young HCQ	0.308 ± 0.170	0.337 ± 0.222	0.326 ± 0.152	0.247 ± 0.072	0.285 ± 0.115
Old HCQ	0.438 ± 0.232	0.561 ± 0.254	0.632 ± 0.238	0.428 ± 0.310	0.637 ± 0.561
Non-classical monocyte (% of CD45)					
Young placebo	1.822 ± 1.230	2.056 ± 1.439	3.336 ± 2.895	2.438 ± 3.217	2.192 ± 2.719
Old placebo	2.268 ± 1.259	3.252 ± 1.584	4.631 ± 3.291	4.957 ± 2.896	4.544 ± 3.432
Young HCQ	1.813 ± 1.382	2.056 ± 1.585	2.433 ± 1.441	1.099 ± 0.909	1.258 ± 1.001
Old HCQ	2.658 ± 2.939	2.308 ± 1.492	2.707 ± 1.819	2.360 ± 1.281	2.469 ± 1.002
Plasmacytoid DC (% of CD45)					
Young placebo	0.416 ± 0.409	0.273 ± 0.162	0.266 ± 0.169	0.242 ± 0.142	0.254 ± 0.204
Old placebo	0.244 ± 0.169	0.200 ± 0.080	0.207 ± 0.123	0.206 ± 0.093	0.283 ± 0.138
Young HCQ	0.212 ± 0.099	0.199 ± 0.114	0.175 ± 0.122	0.183 ± 0.111	0.197 ± 0.125
Old HCQ	0.260 ± 0.612	0.175 ± 0.060	0.219 ± 0.091	0.201 ± 0.119	0.244 ± 0.067

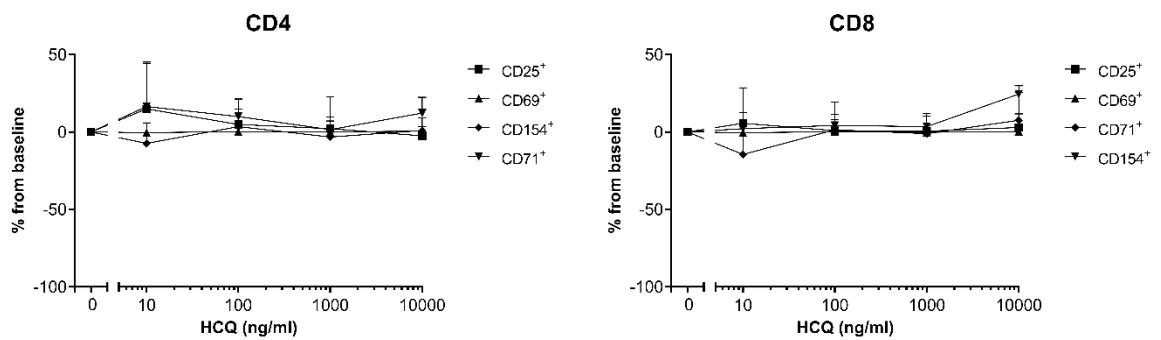
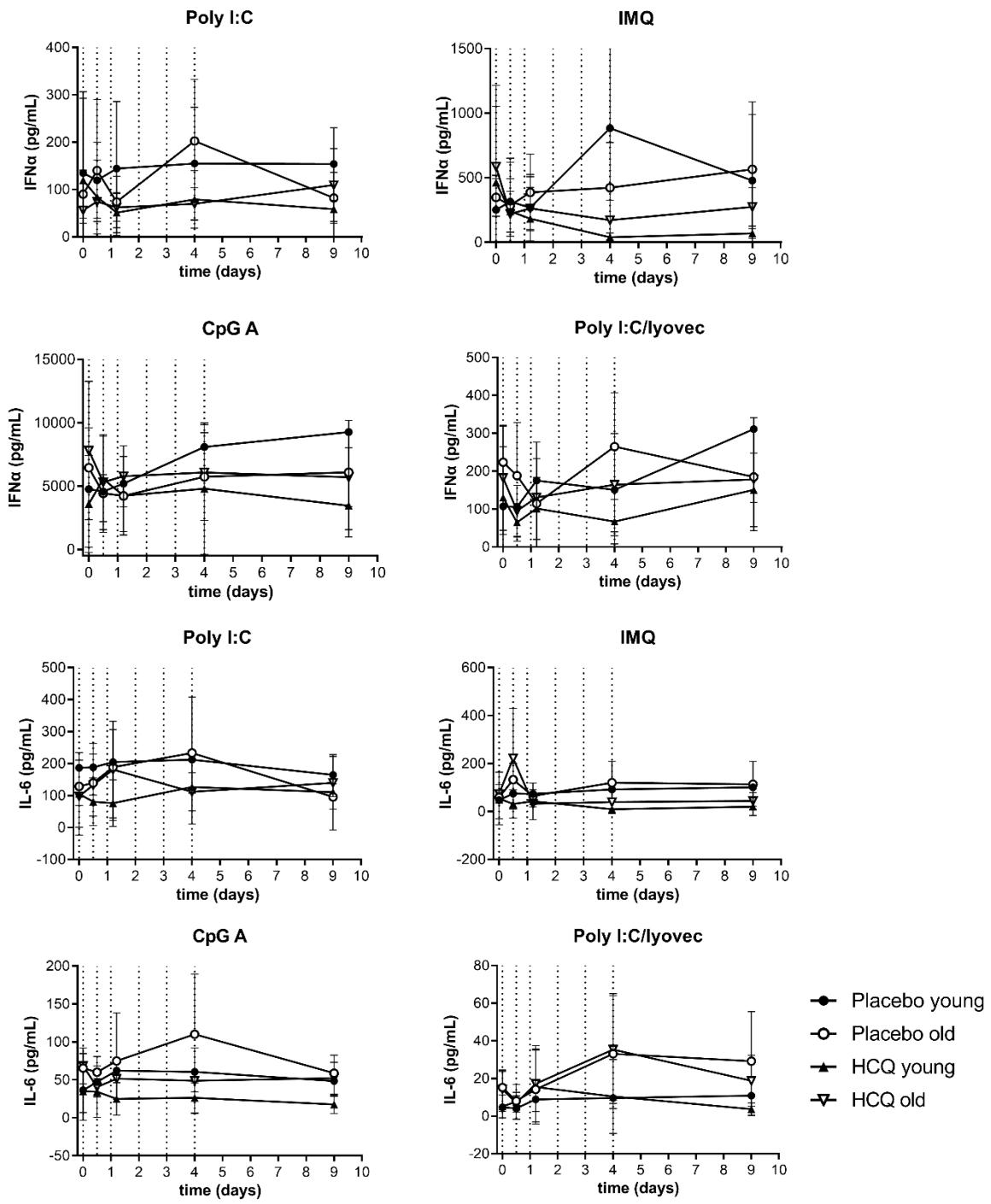


Figure S1: HCQ does not inhibit T cell activation in vitro

Activation markers CD25, CD69, CD154 and CD71 were measured by flow cytometry on CD4⁺ T cells (left panel) and CD8⁺ T cells (right panel), after whole blood stimulation with PHA for 6 hours in the presence of hydroxychloroquine (HCQ). Change from baseline is shown, mean and SD of 6 healthy donors.



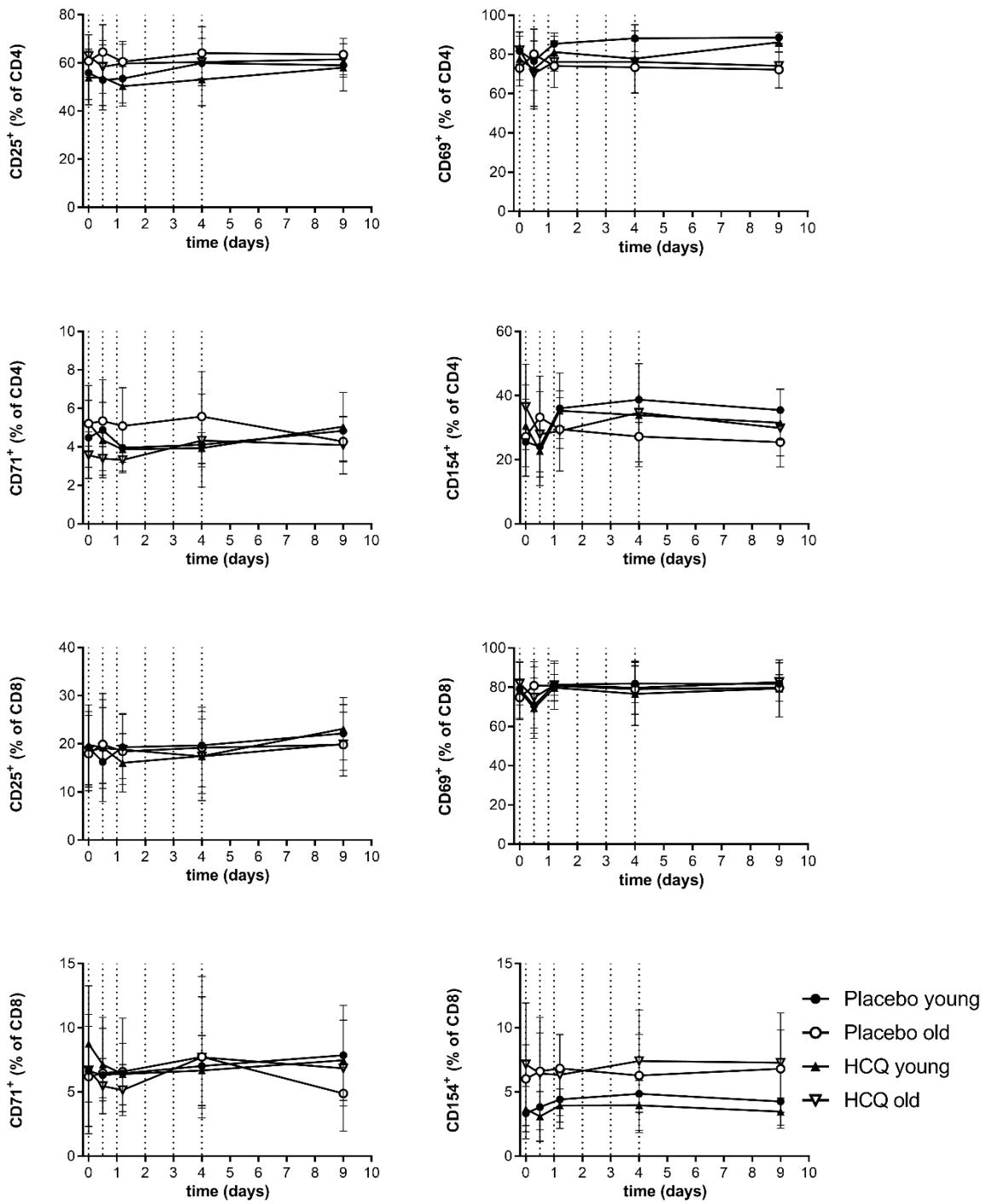


Figure S3: HCQ does not inhibit T cell activation *in vivo*

Activation markers CD25, CD69, CD154 and CD71 were measured by flow cytometry on CD4⁺ T cells (left panel) and CD8⁺ T cells (right panel), after whole blood stimulation with PHA for 6 hours. Data is shown as mean + SD. Dotted vertical lines indicate HCQ dosing times.

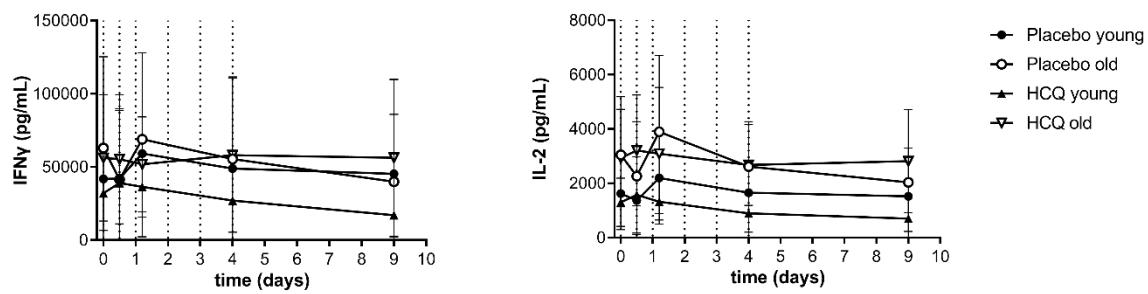


Figure S4: HCQ does not inhibit IFN γ and IL-2 release after ex vivo T cell stimulation

IFN γ (left) and IL-2 (right) release was measured by ELISA after whole blood incubation with PHA for 6 hours. The mean \pm SD are shown of the treatment and age groups. Dotted vertical lines indicate HCQ dosing times.

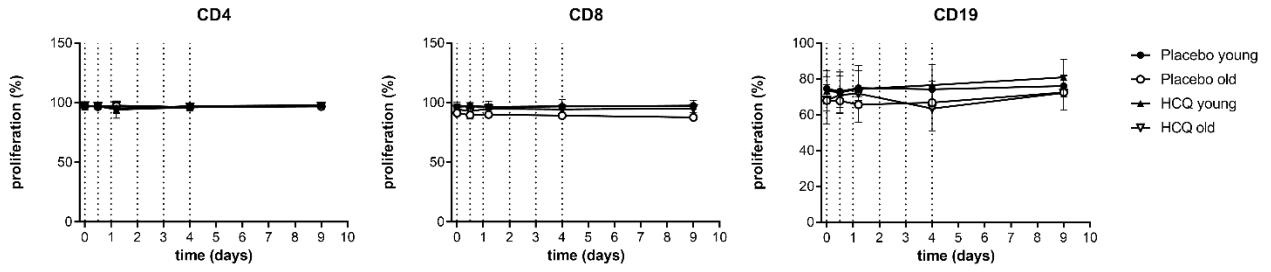


Figure S5: In vivo HCQ effect on T and B cell proliferation, split for age groups.

PBMCs were stained with CTV and stimulated for 5 days with 5 μ g/ml PHA for T cell proliferation (A), or 5 μ g/mL anti-CD40 mAb + 2.5 μ M CpG B for B cell proliferation (B). Proliferation was measured by flow cytometry. The mean \pm SD are shown of the treatment and age groups. Dotted vertical lines indicate HCQ dosing times.